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Benefits and Limitations of Using Hydrochars from Organic Residues as Replacement for Peat on Growing Media

Gianluigi Farru [1,*], Chau Huyen Dang [2], Maja Schultze [2], Jürgen Kern [2], Giovanna Cappai [1] and Judy A. Libra [2,*]

[1] Department of Civil and Environmental Engineering and Architecture, University of Cagliari, Via Marengo 2, 09123 Cagliari, Italy; gcappai@unica.it
[2] Department of Post Harvest Technology, Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB), Max-Eyth-Allee 100, 14469 Potsdam, Germany; hdang@atb-potsdam.de (C.H.D.); mschultze@atb-potsdam.de (M.S.); jkern@atb-potsdam.de (J.K.)
* Correspondence: gianluigi.farru@unica.it (G.F.); jlibra@atb-potsdam.de (J.A.L.)

Abstract: New technologies for the production of peat-substitutes are required to meet the rising demand for growing media in horticulture and the need to preserve natural peatlands. Hydrothermal conversion of organic residues into char materials, hydrochars, with peat-like properties may produce such substitutes, reducing environmental impacts and CO_2 emissions from improper management. To assess their potential as a component in growing media, cress seed germination tests are used to assess hydrochars from digestate (D), spent coffee grounds (SCG), and grape marc (GM). Pre- and post-treatments (extraction, washing, and drying) are applied to remove phytotoxic compounds associated with process waters retained on the hydrochars, and a nitrification bioassay with process water is used to predict their toxicity. All hydrochars achieve similar or better germination results compared to their feedstock, showing a potential to replace at least 5% of peat in growing media. SCG and GM hydrochars show inhibition above 5%, while all post-treated D-hydrochar mixtures produce >3 times longer roots than the control. The nitrification test shows a high sensitivity and good agreement with the high inhibition trends found in the germination tests with process water. Such tests can be a good way to optimize process combinations for the hydrothermal production of peat replacements.

Keywords: biochar; hydrothermal carbonization; waste valorization; germination; peat substitutes; process water

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1. Introduction

Annually, large amounts of growing media are produced to grow a wide variety of plants, including vegetables, fruits, floriculture ornamentals, trees, and shrub ornamentals. A very well-known substrate in growing media is peat, characterised by a porous structure able to provide an optimal amount of air and water to promote the germination of seeds as well as the healthy growth of plant roots [1]. However, natural peatlands play an important role for carbon sequestration and as habitat for many different living species, and their exploitation is not sustainable [2,3]. Therefore, new peat-reduced or even peat-free materials in the growing media are necessary [4,5]. Many researchers have been investigating the feasibility of using renewable sources such as organic residues generated from agricultural and industrial activities as peat replacements. The most important substrates that have already been studied in growing media are compost, bark, and fibres [6]. Among the different materials tested so far (pyrolyzed tomato plants, rice husks, shrimp-derived chitin [7], date palm residues [8], pyrochar from wood [9], and pyrochar from green waste [10]), hydrothermal carbonised char seems to be particularly suitable since it possesses peat-like characteristics that make it a feasible candidate as an additive or even substitute for peat in growing media for horticulture [11,12].

Hydrothermal carbonised char (hydrochar, HC) can be produced from a variety of wet organic materials through the hydrothermal carbonization process (HTC) using water under subcritical conditions [13,14]. Besides the hydrochar, the HTC process also produces a liquid phase (process water, PW) and a small amount of gas. The solid phase shows similarities with materials such as peat or lignite and often has a high nutrient content that makes it feasible for use as a soil improver [15,16]. In addition, several studies showed that the solid products of thermal treatment positively impact the carbon balance of soil, working as a carbon sink in the concept of carbon farming [17,18]. The HTC-process water is generally enriched in nutrients and a wide range of soluble organic compounds, such as phenols, furfurals, organic acids, etc., resulting from the degradation of sugars, lignin, carbohydrates, etc. that occur during the HTC process [19–22]. Some of these compounds may have toxic effects on living species [19,20]. After HTC, the separated hydrochar can retain high amounts of this process water with a water content ranging from 50–85%, depending on the separation process. Thus, the wet hydrochar can hold high concentrations of organic and inorganic compounds, many of which may be phytotoxic [23]. Therefore, post-processing of chars is often necessary before soil application. That can be heating, leaching, or washing, aging, pelletizing, or composting [11,16,24]. In addition, environmentally sound applications for the process water (e.g., fertigation for nutrient recycling [25], biogas production [26] or treatment in wastewater systems [27]) are required.

The addition of each pre- and post-processing step increases the capital and energy costs for production systems; therefore, rapid tests and indicators are needed that can be used to determine appropriate process conditions and steps in the search to produce materials for growing media. Various assessment indicators have been developed to characterise the quality of char materials, ranging from limit values for lumped and individual pollutants using standardised physio-chemical analyses [28,29] to soil function characteristics such as carbon stability and ionic exchange capacity [30] to estimates of ecotoxicity using bioassays [29,31]. Bioassays can be especially useful in providing guidance for application rates to specific soil or crop types [32]. Char materials can directly affect plant performance by decreasing seed germination and/or plant growth, or indirectly by influencing the soil structure and microbiome; the nitrogen cycle is especially sensitive to the presence of nitrification inhibitors [33]. The seed germination bioassay is often used as an early indicator of the effects of char quality on plant performance since, if successful, it is an essential first step for plant growth and development [34,35]. The germination of plant seeds is primarily based on the initiation of growth from the dormant, but still largely undifferentiated embryo [36–38]. It occurs if optimal conditions are provided, such as the presence of water and oxygen, a minimum temperature [36,39] and, in the case of light-sensitive seeds, photoinduction. Various methods have been studied using a variety of seed types (lettuce, spring barley, cress, tomatoes, etc.) in homogenous mixtures (soil/hydrochar in pots [23], soil/pyrochar in shallow germination boxes [34] and in petri dishes [40], soil/hydrochar in plastic containers [16] and hydrochar in petri dishes [41]), on moist substrate wetted with an aqueous solution (water extract from feedstock and pyrochar on filter paper [35]; single compounds on cotton pads or HTC process water in sand [23]; HTC process water in agar medium [25]) or suspended on moist filter paper over the material to test for volatile compounds [23,42]. In addition, various bioassays are available to measure the inhibition of nitrifiers in soil [43,44] and in water systems [45,46].

Apart from recommendations by the European Biochar Certificate [28], to date, specific regulations for using char for agricultural purposes in European countries are lacking. However, as the demand for growing media is increasing, EU regulations on fertilisers have been recently updated, allowing the use of organic products derived from pyrolysis and gasification processes [47,48]. This upcoming modification could boost the valorization of organic residues for soil applications. However, a wide variety of thermal conversion processes besides pyrolysis and gasification can produce potentially beneficial char materials [49]. In this framework, more research is needed to assess the real benefits and

limitations of the use of a variety of chars in growing media in order to widen the spectrum of appropriate feedstocks and production processes for char materials.

This study assesses the feasibility of using hydrothermally treated carbon-rich material derived from the following three common organic residues in Europe: manure digestate, spent coffee grounds, and grape marc, as a peat substitute in plant growing media. Furthermore, the potential benefit of some pre- (extraction of ethanol:water soluble substances, such as phenolic compounds from grape marc) and post-treatments (washing and drying) to remove organic compounds from the hydrochars that may affect plant performance were also analysed. Standardized plant and bacteria-based bioassays were selected for their ease, sensitivity, and speed in assessing toxic effects on living organisms using both hydrochar and process water. Standard cress germination tests using peat were chosen as the most appropriate to determine the inhibitory effects of the hydrochars and their associated process water on germination and subsequent root growth (EN 16086-2:2012). These germination results are compared to those from an aqueous bioassay using aerobic nitrifying bacteria with the process water to assess its potential to predict the possible toxicity related to hydrochar and whether post-treatment is required for the hydrochar.

2. Materials and Methods

2.1. Feedstocks and HTC by-Products

In this study, three different feedstocks were treated via the hydrothermal carbonization process, including the following: (1) separated solid digestate (D) from cow manure collected from a biogas plant (Dobbrikow, Brandenburg, Germany), (2) spent coffee grounds (SCG) collected from a local coffee shop at the University of Cagliari (Italy), and (3) grape marc (GM) from a winery in southern Sardinia (Italy). Feedstocks such as D and SCG were used as they were collected without pre-treatment. Two types of GM feedstock were studied; GM without pre-treatment was compared to GM extracted in an ethanol solution (GM_Ext), as described in Perra et al. (2021), to evaluate the effect of a pre-treatment to remove phenolic compounds from GM before HTC [50].

For the HTC tests, the solid content was set at 13% for D and 10% for SCG, GM, and GM_Ext. Digestate was treated in an 18.9 L pressurised reactor (Parr, Model 4557) in the facilities of ATB—Leibniz Institute (Potsdam, Germany) at the temperature of 240 °C and a holding time of 1 h. The conversion of SCG, GM, and GM_Ext was carried out in a 1.5 L reactor (Berghof, BR-1000) at the laboratory of the University of Cagliari (Cagliari, Italy) at the temperature of 220 °C for 1 h. For the HTC conversion in the 1.5 L reactor, three tests were conducted at the same process conditions to ensure reproducibility and to recover enough carbonised material for the subsequent germination tests.

After each HTC run, the gas formed was released, and the carbonised slurry was separated through a filter press into the solid (HC) and the liquid phase (PW). The process water was filtered at 0.45 µm and stored in plastic bottles at 4 °C for further characterization. Hydrochar from GM and GM_Ext were dried at 105 °C after separation from process water and stored in plastic bags (GM-HC-Dried, GM_Ext-HC-Dried). Hydrochar from D and SCG was washed two times with 30 °C water as post-treatment in order to reduce the amount of organic soluble compounds that may be toxic for plants. In each washing step, water was added to the hydrochar and stirred for 5 min, then it was separated into the two phases and the steps were repeated. In general, water mass three times the mass of solid was used to wash the hydrochar. Wet washed hydrochar from D and SCG was divided into two parts. One part was dried in oven at 105 °C until a constant weight was achieved and stored in plastic bags at room temperature (D-HC-WDried, SCG-HC-WDried), while the remaining part was stored wet in vacuumed bags at 4 °C (D-HC-WWet, SCG-HC-WWet).

2.2. Products Characterization

Feedstock, hydrochars, and process water were physically and chemically characterized. In accordance with the standard methods (ASTM D5373-16 and ASTM D7582-15), the content of C, H, N, S, O, and ash was evaluated to describe the composition of the material

and the modifications that occurred during the HTC process. pH and electrical conductivity (EC) were measured according to EN 13037:2012 and EN 13038:2012, respectively. Moreover, total organic carbon (TOC), chemical oxygen demand (COD), total amount of phenols and concentration of acetic acid were measured for the process water samples. Total organic carbon was evaluated through a TOC analyser (Shimadzu—TOC-VCSH) while COD was measured with the standard titration method after sulfuric acid digestion of samples at 150 °C for 2 h. The COD and TOC values were used to calculate the COD/TOC ratio to assess the average oxidation state of the organics present in the process water. Colorimetric determination of phenols was applied using Folin–Ciocalteu reagent with a UV-spectrophotometer (U-2000, Hitachi, Tokyo, Japan) and the absorbance of the samples was measured at 765 nm. Lastly, acetic acid concentrations were measured through GC-FID (7890B, Agilent Technologies, Santa Clara, CA, USA). As described in Ficara and Rozzi (2001), the acute toxicity of process water on ammonium-oxidizing bacteria in activated sludge was assessed using a pH-stat titration unit (ANITA, Ammonium NITrification Analyser, manufactured by Austep, Milan, Italy) [45]. This titrimetric method assesses the nitrifying activity based on the stoichiometric relationship between ammonium oxidation and acidity production. Test was carried out on an unacclimated activated sludge collected from a municipal wastewater treatment plant (Oristano, Italy). The maximum nitrifying activity was initially measured in the presence of ammonium as the only substrate and used as reference. Increasing volumes of process water were subsequently added to the sludge. The resulting nitrifying activity was compared with the reference and the corresponding inhibition was determined for each dosage. Each test lasted a total of 4 h. At the end of the test, the IC_{50} (the dose that inhibits 50% sludge activity) was estimated.

2.3. Germination Tests

Different germination tests were carried out according to the European standard EN 16086-2:2012. Tests using the contact method for the solid substrates and the extraction method for the process water were performed.

2.3.1. Contact Method for Solid Substrates

The germination tests were carried out on cress seeds (*Lepidium sativum* L.) using Sphagnum peat as reference material according to the standard. The tests with the solid substrates (feedstock, hydrochar) were performed at three different mixtures of peat and solid 5%, 25%, and 50% (wt%, FM); 100% peat was used as a control. An overview of the samples tested, post-treatments used, and moisture content of the fresh mass is given in Table 1. The hydrochars from D and SCG were first washed, and tests were made with wet and dried hydrochar, while hydrochar from GM and GM_Ext was dried with no washing step.

After the preparation of the mixtures, the water content was adjusted with the fist test to have the proper moisture content (60–70wt%) as described by the standard norm (EN 16086-2:2012). Electrical conductivity was checked to be <80 mS/m and pH to be in the range 5.5–6.5. Samples with lower pH were adjusted by adding $CaCO_3$ and left to stabilise for 24 h, while EC was found to be in the required range. Around 60 g of material was placed into squared Petri dishes (100 × 100 × 18 mm) to completely fill them, and 10 seeds were sown on the top of the Petri dishes with one drop of water for each. Petri dishes were closed, sealed with parafilm, and incubated in the dark at 25 °C for 72 h. Three replicate Petri dishes were used for each sample. After 72 h, the Petri dishes were opened, and the germination rate (GR) (the ratio of the number of germinated seeds in the sample to the number germinated in the control) and the length of the roots (RL) were determined for each sample. The average germination rate (AGR) and root length index (RI) for the three replicates were calculated according to Equations (1) and (2) below. In addition, the Munoo-Liisa vitality index (MLV) was calculated according to Equation (3).

$$\mathrm{AGR}[\%] = \frac{\mathrm{GR}_1[\%]\ \mathrm{GR}_2[\%]\ \mathrm{GR}_3[\%]}{3} \quad (1)$$

where GR_1, GR_2, and GR_3 are the germination rates in each replicate.

$$RI[\%] = \frac{\left(\frac{RL_1}{RL_C} + \frac{RL_2}{RL_C} + \frac{RL_3}{RL_C}\right)}{3} \times 100 \quad (2)$$

where RL_1, RL_2, and RL_3 are the average root length in each replicate while RL_C is the average root length in the control samples.

$$MLV[\%] = \frac{(GR_1RL_1) + (GR_2RL_2) + (GR_3RL_3)}{3(GR_CRL_C)} \times 100 \quad (3)$$

where GR_C is the average germination rate in the control.

Table 1. Overview of samples tested.

Material/Code		Pre-/Post-Treatment	Water Content [wt%]	Mixing Ratio between Material and Peat [wt%, FM]			
	Peat	none (used as it is)	74.42	-	-	-	100 (used as control)
Feedstocks	D	separated at biogas plant	70.87	5	25	50	100
	SCG	none (used as it is)	57.85	5	25	50	100
	GM	none (used as it is)	6.17	5	25	50	100
	GM_Ext	extraction in ethanol solution	7.40	n.t	n.t	n.t	n.t
Hydrochars	D-HC	washed after HTC	83.59	5	25	50	n.t
		washed and dried	0 *	5	25	50	n.t
	SCG-HC	washed after HTC	70.54	5	25	50	n.t
		washed and dried	0 *	5	25	50	n.t
	GM-HC	after HTC	66.40	n.t	n.t	n.t	n.t
		dried	0 *	5	25	50	n.t
	GM_Ext-HC	after HTC	69.20	n.t	n.t	n.t	n.t
		dried	0 *	5	25	50	n.t
Materials		**Pre-/Post-Treatment**	**TOC [g L^{-1}]**	**Dilution Ratio (Process Water:Distilled Water) [wt%]**			
Process water	SCG-PW	diluted with distilled water	10.85	0	10	50	100
	GM-PW	diluted with distilled water	9.21	0	10	50	100
	GM_Ext-PW	diluted with distilled water	8.17	0	10	50	100

* Samples were dried at 105 °C for 24 h. n.t: not tested; TOC = Total organic carbon; FM = Fresh matter.

2.3.2. Extraction Method for Liquid Substrates

To assess the possible exploitation of the HTC process water as soil fertiliser and to better understand inhibition effects caused by phytotoxic compounds, process water and process water diluted (1:2 and 1:10) from HTC tests on SCG, GM, and GM_Ext were used as liquid substrates in germination tests using the extraction method. Squared Petri dishes (100 × 100 × 18 mm) were completely filled with perlite (particle size < 2.5 mm) and covered with filter paper (Whatman). 50 mL of process water and diluted process water were poured on the filter, 10 seeds were placed on the top, and the Petri dishes were closed with parafilm. The same procedure for incubation and measurement used in the contact method was applied to the extraction method.

2.3.3. Statistical Analyses

Results of root length and germination rate were validated through statistical analyses (one-way ANOVA and Tukey's HSD test) using the JMP software by SAS to assess the significance of the obtained measurements.

3. Results and Discussion

3.1. Properties of Selected Substrates: Feedstocks, Hydrochar, and Process Water

Results of the elemental characterization (C, H, N, O, S, and ash contents) of the materials used in this work, feedstocks, the produced hydrochars, and peat, are shown in Figure 1. The carbon content was higher in all hydrochars compared to their feedstocks

(an increase of 12.6–16.7%) and also peat (an increase of 5.4–13.1%) as a result of the HTC treatment, while ash and oxygen content were generally reduced. Using the Van Krevelen diagram (Figure 2), where the materials are represented in terms of their atomic ratios of H/C and O/C, the degree of carbonization of the materials can be evaluated. Due to the higher loss of hydrogen and oxygen relative to carbon in the hydrothermal treatment, the values for hydrochar are lower than those of the feedstock, closer to the values for more condensed materials such as lignite and anthracite coal. The anaerobically digested cow manure already starts closer to peat and the D-HC produced at more severe HTC conditions (240 °C) has the lowest values, bringing it closer to the range commonly found for pyrochars. The other hydrochar occupies the lower region that is commonly referred to as peat-lignite. This modification towards peat-like and lignite-like properties may be useful for soil application, especially in terms of seed germination, since the use of the original biomass on soil may promote the proliferation of microorganisms that can be competitors of seeds in the germination phase [51].

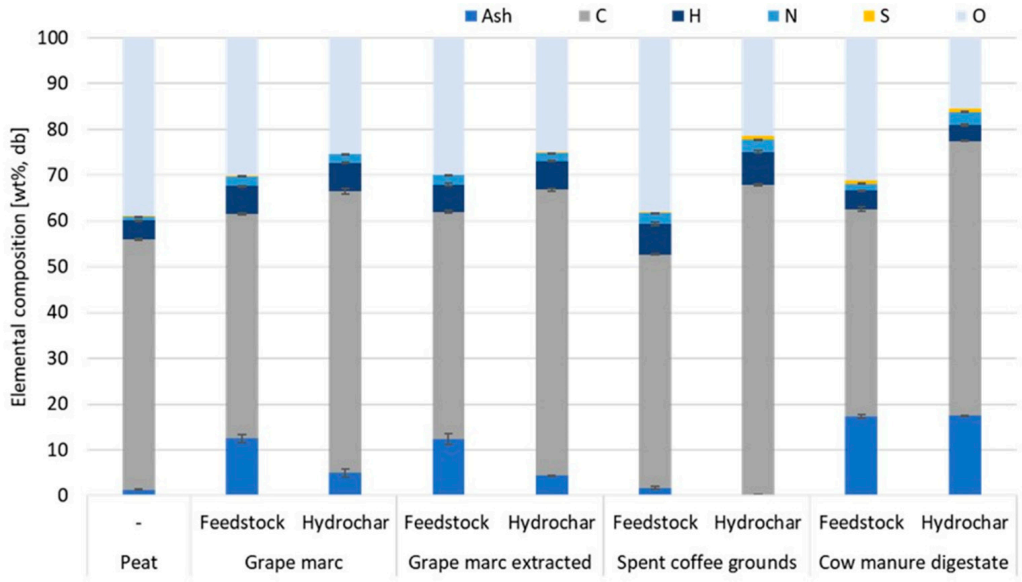

Figure 1. Distribution of ash and macro-element content (wt%, db—dry basis) in peat, HTC feedstock, and resulting hydrochars.

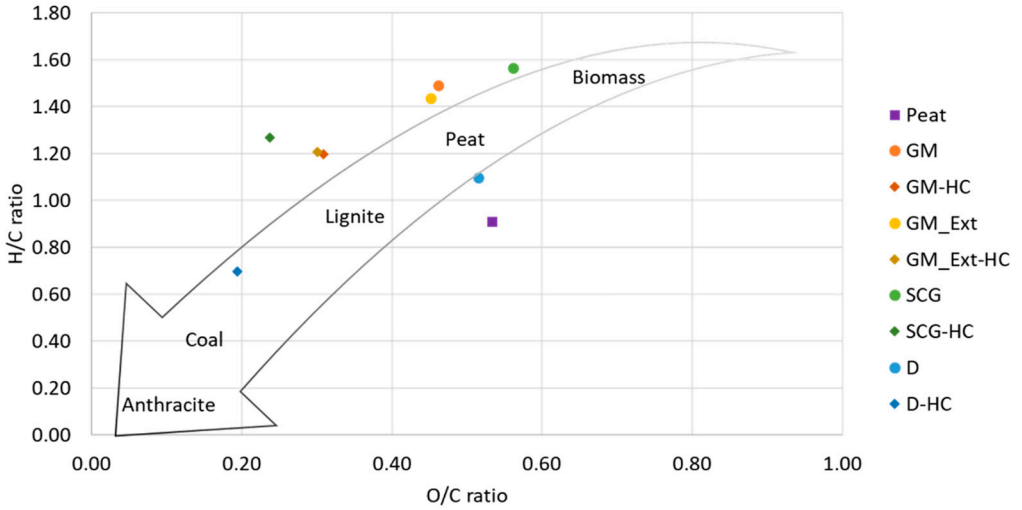

Figure 2. Van Krevelen's diagram of atomic ratios H/C and O/C for the feedstocks and hydrochars with reference regions for typical organic materials [19,21].

Hydrothermal treatment also shifted the C:N ratios in the solids. Although the range remained similar for the feedstocks and hydrochars, with values from 21 to 41, the effect was different depending on the feedstock. The C:N ratio decreased for digestate from 31 to 21, while this was reversed for SCG, GM, and GM_Ext (Table 2). This ratio is generally used to estimate the relative carbon and nitrogen availability for plants. When the C:N ratio is too high (>35), it results in microbial N-immobilization [52]. The lower the C:N ratio, the faster nitrogen is released onto the soil for plant uptake [53,54]. Most of the C:N ratios for the hydrochars lie close to the range optimal for plant growth (20–30) [53].

Table 2. C:N ratio of feedstocks and hydrochars.

Material	D	D-HC	SCG	SCG-HC	GM	GM-HC	GM_Ext	GM_Ext-HC
C:N	31.47	20.90	21.36	25.25	23.56	33.30	23.75	37.18

The process water associated with the hydrochars from SCG, GM, and GM_Ext was characterised by high TOC and COD, acidic pH, and high concentrations of acetic acid and total phenols (Table 3). The high amount of dissolved organic compounds is likely to be inhibitory to many microorganisms and plants. Many aqueous tests are available to study the toxic effects of aerobic bacteria. In this study, a common test of the acute toxicity of process water on nitrifying bacteria was carried out as an indicator for the treatability of the process water in aerobic wastewater treatment plants and/or whether it is safe to use the process water on soils to recycle nutrients. In Table 3, the results of the acute toxicity are expressed as the inhibiting dose for 50% of the microbial population in mL per L of nitrifying sludge (IC_{50}). The lower the value for IC_{50} is, the higher the inhibitory effect. Of the three process waters, GM had the highest inhibitory effect, more than 14 times stronger than that of SCG.

Table 3. Characterization of process water samples from the hydrothermal carbonization of the feedstocks (standard deviations in brackets).

PW Samples	pH	EC [mS cm^{-1}]	TOC [g L^{-1}]	COD [gO_2 L^{-1}]	Total Ph [g L^{-1}]	Acetic acid [g L^{-1}]	IC_{50} [mL L^{-1}]
D	5.34	8.79	11.72	35.00	N.D.	2.36	N.D.
SCG	4.03	3.74	10.02 (±1.16)	29.58 (±0.48)	1.92 (±0.03)	2.01 (±0.04)	8.21
GM	4.37	12.53	10.20 (±0.02)	29.78 (±0.02)	1.32 (±0.02)	2.64 (±0.12)	0.58
GM_Ext	4.26	11.71	8.64 (±0.03)	26.47 (±0.05)	1.52 (±0.02)	2.87 (±0.15)	2.02

EC = Electrical conductivity; TOC = Total organic carbon; COD = Chemical oxygen demand; Total Ph = Total phenols; IC_{50} = Inhibiting dose for 50% of the microbial population; N.D. = not determined.

3.2. Effect of Feedstocks and Hydrochars on Seed Germination and Root Length

The effect of the different feedstock and hydrochar mixtures on the germination of cress seeds is shown in Figure 3. The average germination rate (AGR), which relates the average values from the individual samples to the 100% peat control, is used to compare the effects. For all three feedstocks, almost no seeds germinated in the Petri dishes filled with only feedstock. However, germination in mixtures with peat was successful, achieving AGRs of 100% for feedstock mixtures of D up to 50%. Feedstock mixtures for both SCG and GM already showed inhibitory effects at 25%. Most hydrochar mixtures achieved high germination rates. The values of AGR were similar for D feedstock and hydrochar, while hydrothermal and post-treatment of SCG improved AGR, reaching 90% of the control germination rates for 50% SCG-hydrochar mixtures for both the wet and dried forms. The treatment of GM, however, did not improve the germination at 50% mixtures of dried hydrochar from GM_Ext and GM, which were inhibited by 37% and 100%, respectively.

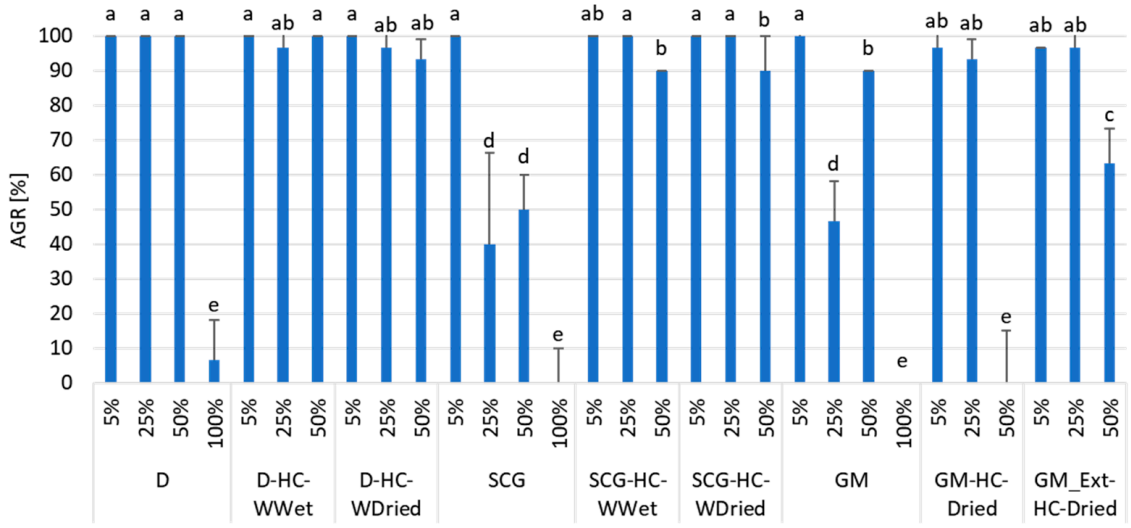

Figure 3. Average germination rates (AGR) assessed for the different hydrochars and their related feedstocks. The results are expressed relative to the peat controls (100%). Samples connected by the same letters are not significantly different.

The average root length of seedlings measured during the germination tests for the feedstocks and hydrochar mixtures is reported in Figure 4a–c. Of the three feedstocks, only mixtures with D promoted root growth, with significantly longer roots in mixtures up to 50% compared to the control. All mixtures of SCG and GM significantly reduced RL, even at the 5% level, although the germination rates were not affected at this mixture. At 25 and 50%, almost no roots developed for SCG and GM, even though some seeds did germinate. In contrast, in Petri dishes with 100% of the three feedstocks, both germination and root growth were almost totally inhibited. Similar inhibition by SCG has been reported by other authors [55], which have attributed this phytotoxic effect to the presence of toxic organic compounds in SCG. Another possible inhibitory effect of the feedstock samples may be due to the mould that developed in those samples with low germination rates. Future tests should clarify whether germination was inhibited due to mould growth or whether mould grew as a result of inhibited germination, e.g., by sterilizing the feedstock before germination tests.

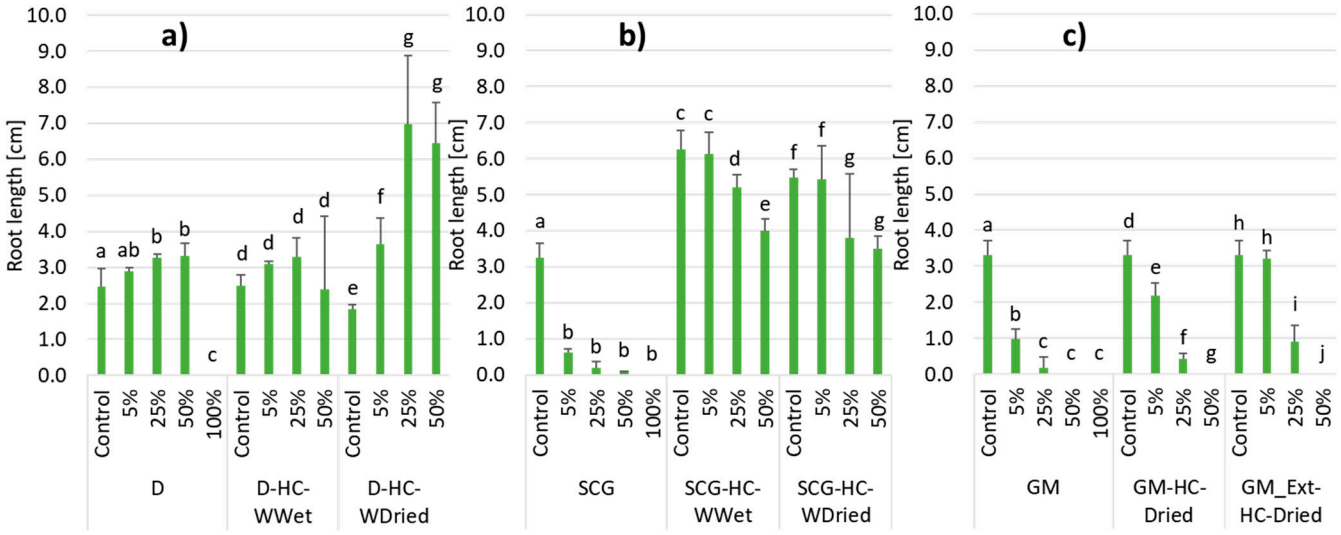

Figure 4. Average root length of seedlings deriving from germination tests using (**a**) D and the related wet and dried hydrochar, (**b**) SCG and the related wet and dried hydrochar, and (**c**) GM and the related dried hydrochar. Samples connected by the same letters are not significantly different.

The hydrothermal treatment of the feedstocks with post-treatment (washing and/or drying) reduced the inhibitory effects of all three feedstocks to varying degrees. For D, RLs similar to the control were achieved for all mixtures of the wet washed hydrochar according to Tukey's HSD test (Figure 4a). In contrast, better RL results were obtained from the Petri dishes using dried washed hydrochar, especially in samples containing 25% and 50% hydrochar. This difference may be attributed to the removal of soluble (via washing) and volatile (via drying) organic compounds through the post-treatments. For SCG, the hydrothermal and post-treatment showed promising results, especially in low percentage mixtures (5 and 25%) (Figure 4b). In fact, all samples with peat replaced by 5% hydrochar achieved results similar to the control. However, higher amounts of hydrochar showed some inhibition in germination (50%) and in root growth (25 and 50%). No benefit was seen from the extra drying step. In contrast, almost all the mixtures of dried hydrochar from GM showed substantial inhibition, even at a low percentage (Figure 4c). Only the 5% mixture of ethanol extracted dried hydrochar (GM_Ext) had root lengths similar to the control. It is likely the presence of phytotoxic compounds, such as phenolic compounds or volatile fatty acids (VFAs, e.g., acetic acid), reduced the germination of seeds and root growth [6,23,56].

The Munoo-Liisa vitality index (MLV), which takes into account both the average germination rate AGR and average root length of the cress seedlings compared to the control [57], was used to evaluate the overall performance. The MLV and RI values for all feedstocks and hydrochar are shown in Figure 5. A reference line at 80% is also shown since some legislation (e.g., the Finnish Act on Fertilizer Products) has set this as a target value for MLV% in cress germination tests [57]. Although values below this target are presumed to indicate the presence of potential phytotoxic compounds in the substrate tested, Maunuksela et al. (2012) found that pig manure, a material conventionally used on soil, had MLV values lower than 80% and suggested that 60% be considered adequate for good product quality [57].

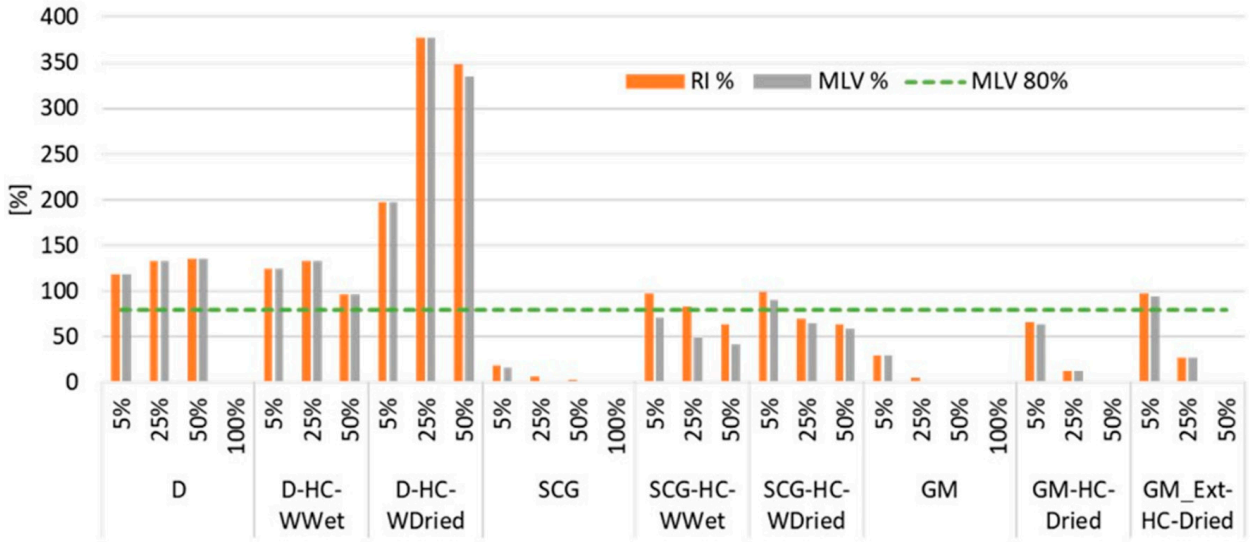

Figure 5. Values of root length index (RI), and Munoo-Liisa vitality index (MLV) calculated for each sample in germination tests with the contact method. The results are expressed relative to the controls (100%).

All the hydrochars in this study achieved better MLV results when compared to their feedstock. Post-treatments had positive effects on both germination and root growth, especially the application of both washing and drying. The D-feedstock and wet washed hydrochar mixtures had MLV values well above the target of 80%, but the washed and dried D-hydrochar achieved a much higher MLV, over 300% at mixtures of up to 50%. Although the MLV for SCG-feedstock and hydrochar mixtures were very much lower than those for D, the trends for the hydrochars were similar. Hydrothermal treatment of SCG with both

washing and drying improved germination, increasing MLV from 16% for the 5% feedstock mixture to 50% and 65% and to 40% and 60% for the 25% and 50% hydrochar-mixtures (wet and dried), respectively. In contrast, only the 5% mixture of hydrochar from GM and GM_Ext achieved similar germination results. Although the pre-treated GM with ethanol extraction produced a carbonised material that showed improved results in comparison with hydrochar produced without pre-treatment, these improvements were effective only when low percentages of hydrochar were used in the growth substrate.

3.3. Effect of Process Water on Seed Germination

Germination tests using the extraction method were carried out with the process waters from the hydrothermal treatment of the three feedstocks (SCG, GM, and GM_Ext) that caused the highest inhibition in the peat-replacement tests discussed previously. Such tests with process water may be helpful to answer questions on whether post-treatment of the hydrochars is advisable before application as a soil amendment or growth media and/or how to treat the process water.

The AGR shown in Figure 6a indicates that all samples of process water from the three feedstocks inhibited germination to some extent. The process water from GM_Ext had the least effect, with AGR ranging from 90% (10% dilution) to 40% (undiluted). No germination was seen for undiluted SCG and GM process water. The effect of the process water was much stronger on the root length (Figure 6b). The root length was decreased in all samples, ranging from almost no growth with undiluted process water to 45–55% shorter roots for the 10% dilution.

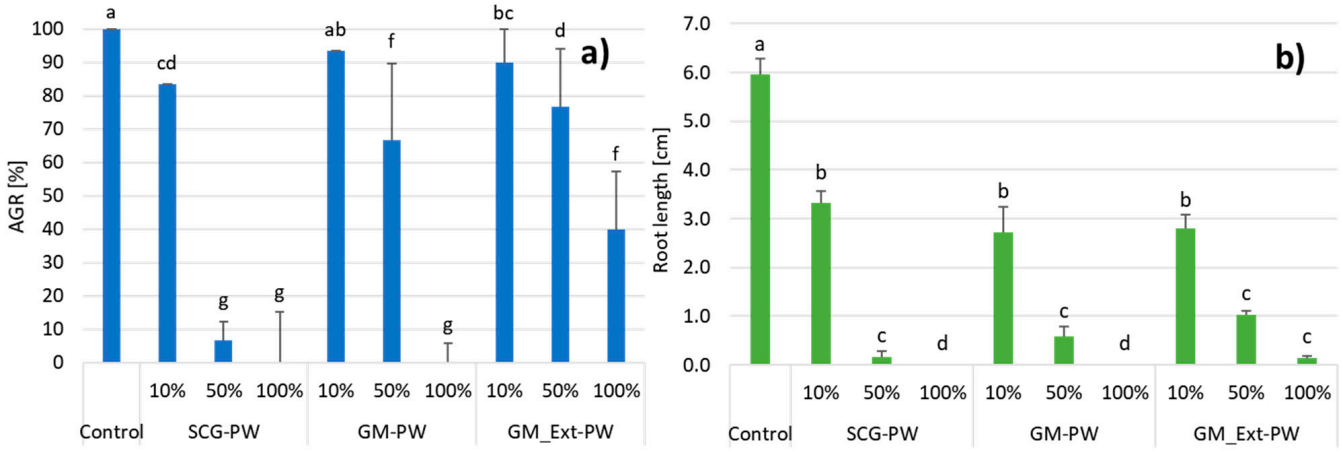

Figure 6. Germination test results using process water for (**a**) average germination rate (AGR) and (**b**) average root length. Samples connected by the same letters are not significantly different.

High inhibition was seen for all three process waters, with MLV decreasing at higher concentrations of process water (Figure 7). The MLV of all samples remained well below the target value of 80%, only reaching 47% for the 10% SCG-process water dilution. The inhibition is likely due to the high amount of organic compounds dissolved in the process water (Table 3). This consideration is supported by different authors [23,58] who found similar inhibition of germination and seedling growth from process water. Process waters typically contain high concentrations of many small molecular organic compounds that may be inhibitory to germination, such as VFAs and other organic acids, as well as furan and phenolic compounds and aldehydes [19,21,23]. The high organic loading of the process water (e.g., 8.64–10.2 gTOC/L; 26.47–29.78 gCOD/L) means that even at a 10% dilution, the seeds are exposed to the equivalent of a high-strength wastewater. Bargmann et al. (2013) identified some specific compounds (guaiacol, levulinic acid, and glycolic acid) that inhibited cress seed germination and three others (acetic acid, glycolaldehyde dimer, and catechol) that negatively impacted growth [23]. They also found that they could use the dissolved organic carbon content in their process water from five feedstocks to

predict germination-inhibiting effects. In Figure 7, it can be seen that in each feedstock, higher TOC concentrations are related to lower MLV. However, comparing the three feedstocks, the small differences between the TOC and acetic acid values in the process water do not correspond to the differences in MLV. The differences in values are also too small for another possible indicator, the COD/TOC ratio, which can be used to assess the average oxidation state of the organics present in the process water, and indirectly indicate the type of compounds present. For example, the value for the three process waters (ca. 3.0 gCOD/gTOC) is more similar to that of phenol than acetic acid (3.1 vs. 2.67 gCOD/gTOC).

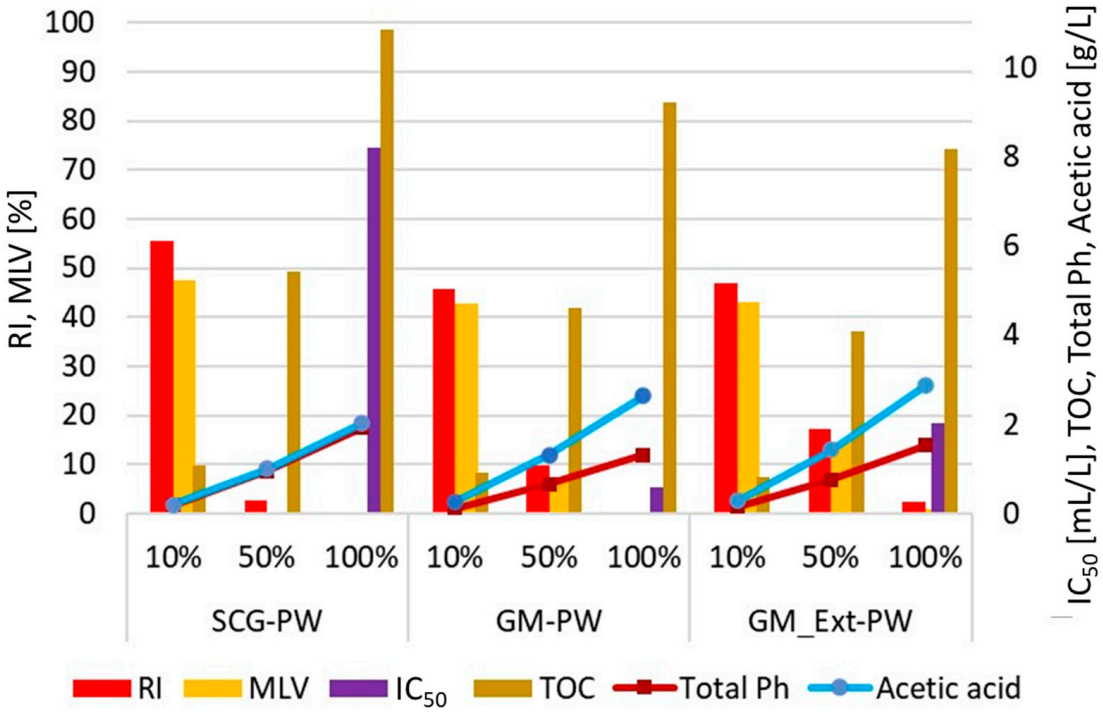

Figure 7. Characteristics of germination tests using process water. Acetic acid and Total phenols (Total Ph) are given in lines and Inhibition dose (IC_{50}), Total organic carbon (TOC), Root length index (RI), and Munoo-Liisa Vitality index (MLV) in bars.

An additional lumped parameter, total phenols (Total Ph), was also measured in the process water (Figure 7), but the values do not follow the trend found in the germination tests. The highest Total Ph concentration was measured for SCG, but SCG has the highest MLV at the 10% process water dilution. It is interesting to note that the pre-treatment for GM_Ext, where mainly polyphenolic compounds are extracted from the feedstock with ethanol, did not reduce the Total Ph measured in the process water compared to GM. Nonetheless, the process water from the GM_Ext showed less inhibition on the seed germination and root growth than the GM-PW. Thus, Total Ph does not seem suitable as an indirect indicator of inhibition for these feedstock process waters.

The trend in the inhibitory effects seen with the MLV values is better matched by the results of the bioassay using aerobic nitrifying bacteria. The feedstock process water with the lowest MLV and IC_{50} (which indicates the highest inhibition) is GM, while SCG has the highest values for MLV and IC_{50}. Nitrifying bacteria are essential in the nitrogen cycle [59], converting ammonia to nitrates in soil and in wastewater treatment plants. Inhibitors for these bacteria can be purposely added to fertilisers to minimise N losses and improve N use efficiency in agricultural ecosystems [60] or stringently avoided to ensure nitrogen removal in wastewater systems [45,46]. The results here show that this aqueous bioassay with ammonium-oxidizing bacteria is very sensitive to inhibitors that affect nitrification as well as germination. Moreover, aqueous bioassays were able to detect inhibition at lower

process water quantities than germination. The bacteria seemed to be more sensitive than plants since the most highly diluted samples in germination, 10% process water, are still more than twelve times more concentrated than the one used measuring IC_{50} value for SCG, and for GM almost 180 times more concentrated. In Figure 8, the germination results for both the solid and liquid products from the HTC of GM and GM_Ext are compared to the IC_{50} measured for the process water. The MLV for the hydrochar and process water both show less inhibition from the pre-treated GM than from the GM, and this corresponds well with the IC_{50} values. Comparing the two tests, the acute toxicity test on nitrifying bacteria is faster than germination tests, considering the time used by the former for the material preparation, incubation (72 h), and root measurement. On the other hand, tests on nitrifying bacteria require 24 h of aeration for the activated sludge preparation and only 4 h to obtain the results. In addition, this kind of test considerably reduces the evaluation effort as the only task required is to dose a certain amount of sample about every 20 min.

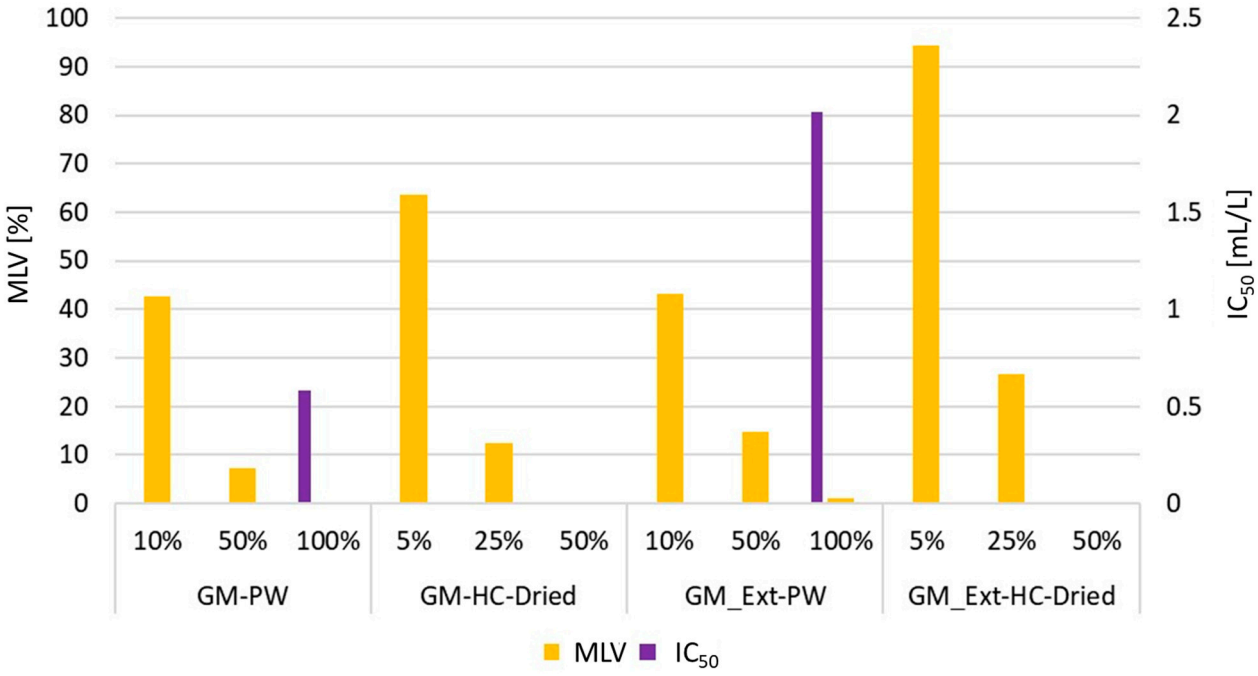

Figure 8. Comparison between MLV (Munoo-Liisa Vitality index) for hydrochar and process water for GM and GM_Ext and the IC_{50} (inhibition dose) for process water.

4. Conclusions

Germination tests on hydrochars from D, SCG, and GM showed that these substrates may be considered as a substitute for peat in growing media. However, the quantity of peat that can be substituted varies according to the feedstock type and the pre-treatments or post-treatments applied. The most promising is the digestate and its hydrochar, as indicated by the root length of the cress seedlings under study. It was found that pretreatment of the feedstock (GM extraction) and post-treatments of the hydrochars (washing and drying) play an important role for improving the quality of hydrochars as reflected by positive effects on germination. In fact, SCG hydrochars improved when both post-treatments were applied, while hydrochar from GM with only the drying step showed high inhibition; however, with pre-treatment, GM_Ext-HC reached almost 100% of the control values at a 5% mixture. The potential of replacing at least 5% of peat in growing media for horticulture might be a substantial contribution to reducing the global use of natural peatlands, although the need for a post-treatment of the hydrochars has to be taken into account. Future studies may prove how far the hydrochar content can be enhanced between 5 and 25%.

The inhibition that affected samples containing a high amount of hydrochar from SCG and GM may be due to the formation of phytotoxic compounds during the HTC process

and the retention of process water on the hydrochar. Therefore, it may be important and necessary to remove the process water, which may contain phytotoxic substances. Initial evaluation of the relatively robust and quick nitrification bioassay on the process waters showed high sensitivity and good agreement with the inhibition trends found in cress germination tests. It was a better predictor than the chemical lumped parameters (TOC, total phenols) for the presence of phytotoxic compounds in the hydrochars and process waters and their possible adverse effects. However, to obtain a better understanding, the accordance between the nitrification bioassay and the germination cress test should be studied in more detail. Such tests can be helpful in developing and optimising process combinations for the hydrothermal production of peat replacements by assessing the potential adverse effects of hydrochar by testing the process water toxicity.

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